

Differences in bacterial composition between vascular epiphyte and parasitic plants living on the same host plants

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Abstract. Do HDK, Luqman A, Vu MT, Nguyen HD, Putro YK, Rofiq EA, Santoso H, Kristanti AN, Hariyanto S, Bui LM, Manuhara YSW, Wibowo AT. 2022. Differences in bacterial composition between vascular epiphyte and parasitic plants living on the same host plants. *Biodiversitas* 23: 5798-5805. Epiphytic and parasitic plants can grow and complete their life cycle while attached to the host. Therefore, the interactions between these plants and their host provide profound evidence for co-evolution. During these symbiotic interactions, bacteria are actively exchanged between parasitic and epiphytic plants with their hosts. Since epiphytes and parasitic plants have different ways of life, they might assemble their bacterial community differently despite living in the same host. However, direct microbiome comparisons between epiphytic and parasitic plants colonizing the same host have never been evaluated. In this study, we examined the bacterial compositions of the epiphytic *Hoya* sp. and parasitic *Dendrophthoe* sp. that grow in two host species, frangipani (*Plumeria* sp.) and teak (*Tectona grandis*). The results revealed that bacterial compositions in the root of *Hoya* sp. are highly similar to the peripheral tissue of the host stem. In contrast, bacterial composition in the haustoria of *Dendrophthoe* sp. is quite distinct from the host. These results revealed that epiphytes and parasitic plants acquire and assemble their microbiome differently, despite living in the same host species. These differences might originate from different nutrient acquisition strategies between the two plants.

Keywords: Epiphytic plants, leaf microbiome, microbial interaction, parasitic plants, root microbiome, species diversity

INTRODUCTION

Plant-associated microbial communities are varied in diversity and composition across different tissues, individuals, and species (Dastogeer et al. 2020; Wu et al. 2020; Vu et al. 2022). Plants and microbes are likely to interact interchangeably; while plants might produce certain metabolites to regulate microbial composition, the microbes may also directly affect the metabolic and metabolism of the host (Pascale et al. 2019; Korenblum et al. 2020). Most studies regarding plant-microbe interaction are conducted in terrestrial plants, while studies in plants with unique ecological niches and lifestyles, such as epiphytic and parasitic plants, are very limited (Sheng-Liang et al. 2014; Iasur Kruh et al. 2017; Cui et al. 2018; Fitzpatrick and Schneider 2020). The microbiome composition in these non-terrestrial plants is likely shaped by a complex system involving the biology and microbial composition of host plants, epiphytes, and parasitic plants. Studies in such a unique system could provide new insight into plant microbiomes' dynamic assembly and function.

Parasitic plants could directly acquire resources and nutrients from their host plant through specialized organs called haustoria. Haustoria attach and penetrate the host

plant's vascular tissue to facilitate water absorption and nutrients (Joel et al. 2013; Yoshida et al. 2016). Besides water and nutrients, haustoria might facilitate the exchange of endophytic microorganisms between the parasitic plants and their host. Iasur Kruh et al. (2017) reported a possible bacterial exchange between the parasitic plant *Phelipanche aegyptiaca* and its tomato host. The *P. aegyptiaca* bacterial community was shifted from Gram-negative to Gram-positive bacteria during their parasitic life cycle in the host. Moreover, they also reported that *Pseudomonas* PhelS10 from tomato root could suppress *P. aegyptiaca* germination, suggesting an interaction between host-associated bacteria and parasitic plants' fitness (Iasur Kruh et al. 2017). Similarly, Fitzpatrick and Schneider (2020) also reported that the bacterial community in parasitic *Orobancha hederarum* might be derived but also distinct from the microbiome of its host plant, *Hedera* spp. (Fitzpatrick et al. 2018). Despite this knowledge, how microbial communities in parasitic plants are established and their role in the parasitic life cycle remains to be elucidated. Furthermore, the microbiome of parasitic plants behaves and assembles across different host species with different immunity, and microbial pools are still unknown.

In contrast to parasitic plants, epiphytes germinate and grow on the surface of host plants without developing parasitic roots. They acquired water and nutrients from the air, rain, debris, and suspended soil that accumulated around the plant (Bartels and Chen 2012). Compared to ground plants, epiphytes acquire better light access and protection against herbivores (Batke 2012). Epiphytes benefit from their host plants, and the overall relationship in most cases seems to be one-directional. The host plants provide substrate above the ground for the epiphytes without benefiting themselves (Callaway et al. 2002). As reported in parasitic plants, the microbial transfer might also occur between the epiphytic plants and their host. Studies in epiphytic orchids reported that the root fungal composition of epiphytic orchids is dominated by Ascomycota and Basidiomycota (Cevallos et al. 2018; Maldonado et al. 2020; Cevallos et al. 2022), while the bacterial composition in the root is dominated by Cyanobacteria (Tsavkelova et al. 2001; Tsavkelova et al. 2003a; Tsavkelova et al. 2003b; Tsavkelova et al. 2022). However, whether the microbial community in the root of epiphytic orchids originated from the host plants is unknown since there is still no study on microbial correlation and interaction between epiphytic plants and their host currently.

Very few studies have investigated the microbial composition in vascular epiphytic and parasitic plants and their respective host. Moreover, a direct comparison between the microbiome of parasitic and epiphytic plants that grow in the same host species has never been conducted. Such a study could give us insight into the dynamic of plant-plant, microbe-microbe, and plant-microbe interactions across species. Here we investigated the bacterial community associated with the parasitic plant *Dendrophthoe* sp. and the epiphytic plant *Hoya* sp. associated with two different host plants: frangipani (*Plumeria* sp.) and teak (*Tectona grandis*). Here we showed that bacterial composition in epiphytic plants, especially in the root, is highly similar to their host, while

bacterial composition in parasitic plants is quite distinct from the host.

MATERIALS AND METHODS

Study area and sample collection

Dendrophthoe sp. and *Hoya* sp. can grow and complete their life cycle while attached to frangipani (*Plumeria* sp.) and teak (*Tectona grandis*). This study collected *Dendrophthoe* sp. and *Hoya* sp. from frangipani and teak trees that grow across various locations in the Mojokerto area, East Java, Indonesia (Supplemental Table 1). Plants were identified based on their morphological characteristics, especially the flower and leaf structures (Figure 1). Leaf and root were collected from three *Hoya* plants attached to frangipani and teak, together with peripheral tissue of the host where the *Hoya* root is attached. Similarly, leaf and haustoria were collected from three *Dendrophthoe* plants attached to frangipani and teak, together with peripheral tissue of the host where *Dendrophthoe* haustoria are attached. All samples were washed using a sterile PBS solution previously described (Edwards et al. 2018) to remove rhizospheric microbes, then stored at -20°C.

DNA extraction and sequencing

For genomic DNA extraction, samples were homogenized to powder using mortar and pestle in liquid nitrogen. DNA was extracted from the homogenized tissue using ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Orange, CA, USA), according to manufacturing instructions. Genomic DNA was quality controlled and then used as input material to prepare amplicon sequencing libraries. In brief, 30 ng of DNA template and 16S rRNA V3-V4 primers were used for Polymerase chain reaction (PCR) according to the Illumina PCR Quantification Protocol Guide (Illumina, San Diego, CA, USA).

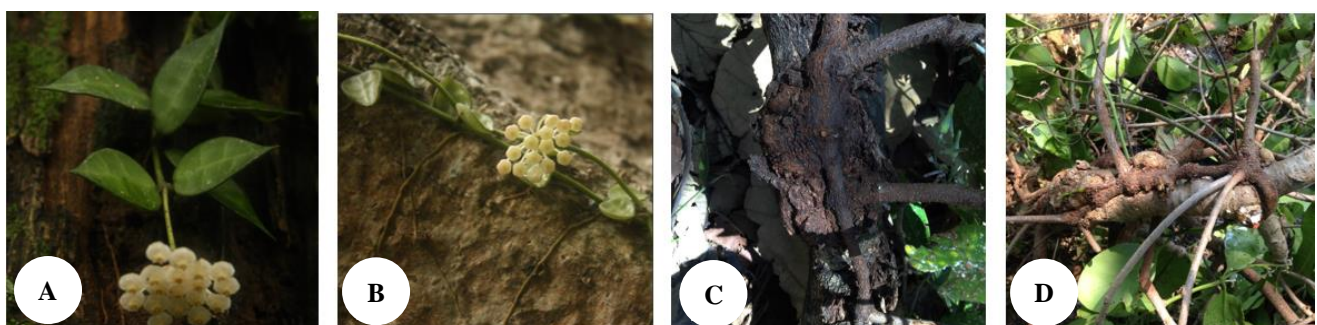


Figure 1. Morphological characters of epiphytic *Hoya* sp. and parasitic *Dendrophthoe* sp. A. The flower and leaf morphology of *Hoya* sp. found on the teak tree, B. *Hoya* sp. found on the frangipani tree, C. Morphology of *Dendrophthoe* sp. found on the teak tree, D. *Dendrophthoe* sp. found on a frangipani tree

All PCR products (approximately 400 bp in length) were purified by Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) dissolved in Elution Buffer and eventually labeled to finish library construction. Library size and concentration were detected by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Qualified libraries were sequenced on HiSeq 2500 platform (Illumina, San Diego, CA, USA) by 2 × 250-bp paired-end runs. The sequencing data will be made publicly available at BioProject PRJNA813337 at NCBI following the publication of the manuscript.

Bacterial composition analysis

The sequencing data were analyzed using QIIME 2 workflow to explore the biodiversity of the surveyed samples (Bolyen et al. 2019). First, the raw data were filtered to generate high-quality clean reads for further analysis with QIIME 2. Specifically, truncate reads whose average Phred quality values are lower than 20 over a 25 bp sliding window will be truncated. Then, the reads whose lengths were shorter than 75% of their original lengths after truncation were removed. Additionally, reads contaminated by adapter sequences and containing ambiguous bases (N base) were eliminated. The low-complexity reads with ten same consecutive bases were also deleted from the data. Then, the clean sequencing data were demultiplexed to remove the primer sequences in the reads after being imported into QIIME 2. To denoise and dereplicate the sequences, DADA2 was used (Callahan et al. 2016). Then the clean data were clustered into groups using the VSEARCH plugin with 99% similarity to the SILVA references after removing chimeras Quast et al. 2013; Rognes et al. 2016). The sample's shared Operational taxonomic unit (OTU) was illustrated using online web tools (<https://bioinformatics.psb.ugent.be/webtools/Venn/>). The sequence data were then classified with SILVA taxonomy data through the classify-sklearn method (Pedregosa et al. 2011). The Shannon diversity index was used to evaluate the alpha diversity of the samples. For

Principal Component Analysis (PCA), the ClustVis web tools were used with default settings (Metsalu and Vilo 2015). The data were deposited to NCBI under accession number PRJNA813337.

RESULTS AND DISCUSSION

Taxonomic composition of bacteria associated with epiphytic, parasitic, and host plants

The assignment of bacterial OTUs revealed that the most abundant phyla across different *Hoya* samples are Proteobacteria, Actinobacteria, and Acidobacteria. Regardless of the host, bacterial composition in the leaf is dominated by Actinobacteria and Proteobacteria, while the bacteria in the root are mainly Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi. The bacterial composition in the root of *Hoya* resembled the one observed in the stem of the host plants, suggesting that the host microbiome strongly influences bacterial composition in the epiphytic plants (Figure 2, Supplemental Table 2).

Bacterial composition among different *Dendrophthoe* organs is more diverse. The leaf microbiome of *Dendrophthoe* hosted on frangipani is only composed of Proteobacteria, while the one associated with teak consisted of Firmicutes, Proteobacteria, Bacteroidota, and Actinobacteria. In the haustoria of *Dendrophthoe* hosted on frangipani, Actinobacteria is detected as the most abundant phylum, while in the one that parasitizes teak, Proteobacteria is more abundant. The bacterial compositions in the stems of frangipani and teak hosting *Dendrophthoe* are similar. They consisted mainly of Proteobacteria, Actinobacteria, Bacteroidota, and Acidobacteria. The diverse bacterial composition across different *Dendrophthoe* samples suggests that bacterial composition in *Dendrophthoe* is influenced by various factors, not only by the microbiome of the host plant (Figure 2, Supplemental Table 2).

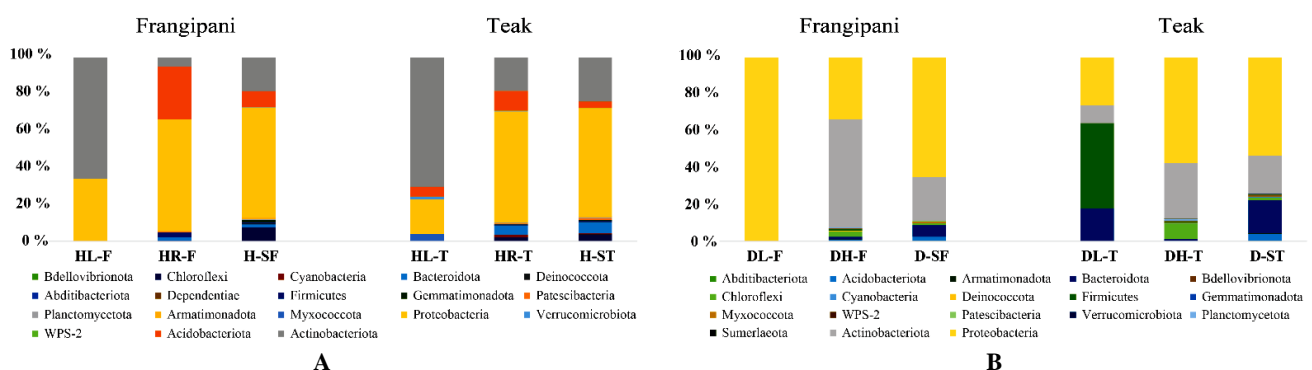


Figure 2. Bacterial taxonomic composition and relative abundance at the phylum level. A. Relative abundance of bacteria associated with *Hoya* sp. hosted on frangipani and teak at phylum level and B. Relative abundance of bacteria associated with *Dendrophthoe* sp. hosted on frangipani and teak at the phylum level. Abbreviations: HL-F: *Hoya* leaf found on frangipani, HR-F: *Hoya* root found on frangipani, H-SF: stem of frangipani hosting *Hoya*, DL-F: *Dendrophthoe* leaf found on frangipani, DH-F: *Dendrophthoe* haustoria found on frangipani, D-SF: stem of frangipani hosting *Dendrophthoe*, HL-T: *Hoya* leaf found on teak, HR-T: *Hoya* root found on teak, H-ST: stem of teak hosting *Hoya*, DL-T: *Dendrophthoe* leaf found on teak, DH-T: *Dendrophthoe* haustoria found on teak, D-ST: stem of teak hosting *Dendrophthoe*

Bacterial diversity and structure in epiphytic, parasitic, and host plants

In all *Hoya* sp. samples, regardless of the host species, the total number of identified OTUs and the highest diversity index are in the stem of the host plant, followed by the root of *Hoya*, and lowest in the *Hoya* leaf. For example, only 4 and 11 OTUs have been detected in the leaves of *Hoya* hosted in frangipani and teak, respectively, compared to 44 and 140 OTUs found in the roots. The results showed that bacterial diversity in the *Hoya* root is much higher than in the leaf, suggesting that a significant portion of bacteria in host stems are transferred to the root but not to the leaf (Table 1).

In parasitic *Dendrophthoe*, the largest number of OTUs and the highest diversity index are detected in the haustoria of *Dendrophthoe*, followed by the stem of the host plant, and the lowest was in the leaf of *Dendrophthoe*. Similarly to *Hoya*, a very low number of OTUs is detected in the leaf compared to the haustoria. In *Dendrophthoe* that parasites frangipani, only 6 OTUs were detected in the leaf

compared to 289 OTUs found in haustoria. In contrast, in plants that parasite teak, only 9 OTUs were detected in the leaf compared to 364 OTUs found in the haustoria. The total number of OTUs and diversity index in the haustoria are even higher than the stems of the host plants, suggesting that the bacterial community in the haustoria is not solely dependent on and derived from the host.

Next, we performed PCA to evaluate the grouping of the samples based on OTUs. PCA clustered the leaf samples of *Hoya* and *Dendrophthoe* together, separating them from the other samples. The haustoria samples of *Dendrophthoe* are also grouped and separated from the other samples, while the root sample of *Hoya* is clustered with the stem of the host plants. This pattern of PCA clustering is consistent across two different host plants (Figure 3). Together, these results show that the bacterial community in the roots of epiphytic plants is highly similar to their host, while the bacterial composition in the haustoria of parasitic plants is quite distinct from the host.

Table 1. Total OTUs and Alpha diversity are sampling populations as measured by Shannon Index

Host	Species	Sample population	Code	Total OTUs	Shannon index
Frangipani	<i>Hoya</i> sp.	<i>Hoya</i> leaf	HL-F	4	0.07
		<i>Hoya</i> root	HR-F	44	0.88
		Stem of frangipani hosting <i>Hoya</i>	H-SF	120	0.93
	<i>Dendrophthoe</i> sp.	<i>Dendrophthoe</i> leaf	DL-F	6	1.81
		<i>Dendrophthoe</i> haustoria	DH-F	289	7.26
		The stem of frangipani hosting <i>Dendrophthoe</i>	D-SF	72	0.68
Teak	<i>Hoya</i> sp.	<i>Hoya</i> leaf	HL-T	11	0.06
		<i>Hoya</i> root	HR-T	140	2.14
		The stem of the teak hosting <i>Hoya</i>	H-ST	452	4.86
	<i>Dendrophthoe</i> sp.	<i>Dendrophthoe</i> leaf	DL-T	9	0.45
		<i>Dendrophthoe</i> haustoria	DH-T	364	6.47
		The stem of the teak hosting <i>Dendrophthoe</i>	D-ST	243	4.89

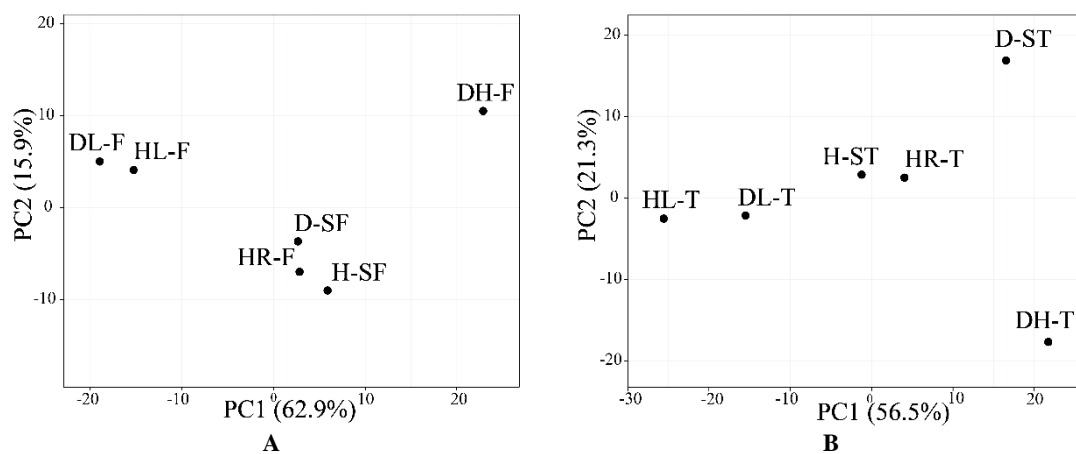


Figure 3. Principal Components Analysis based on OTUs associated with *Dendrophthoe* sp. and *Hoya* sp. hosted at A. frangipani and B. teak. Abbreviations: HL-F: *Hoya* leaf found on frangipani, HR-F: *Hoya* root found on frangipani, H-SF: stem of frangipani hosting *Hoya*, DL-F: *Dendrophthoe* leaf found on frangipani, DH-F: *Dendrophthoe* haustoria found on frangipani, D-SF: stem of frangipani hosting *Dendrophthoe*, HL-T: *Hoya* leaf found on teak, HR-T: *Hoya* root found on teak, H-ST: stem of teak hosting *Hoya*, DL-T: *Dendrophthoe* leaf found on teak, DH-T: *Dendrophthoe* haustoria found on teak, D-ST: stem of teak hosting *Dendrophthoe*

Shared OTUs between parasitic, epiphytic, and host plants

The results from the PCA analysis are reflected in the number of shared OTUs between the parasitic, epiphytic, and their respective host plants. A large percentage of the OTUs found in *Hoya* roots are shared with the stems of host plants. We found that 72.7% and 67.85% of OTUs found in the root of *Hoya* were also detected in the stems of frangipani and teak, respectively. On the other hand, only 11.42% and 25.34% of OTUs found in the haustoria of *Dendrophthoe* were detected in frangipani and teak stems. A similar pattern is observed in the leaf. *Hoya* leaf shares a high percentage of its OTUs with the host plants (100%, and 63.63% with stems of frangipani and teak, respectively).

On the contrary, a low percentage of OTUs are shared between *Dendrophthoe* leaf and their host (16.66%, and 11.11% with frangipani and teak stem, respectively) (Figure 4). Together, these results indicate that the bacterial composition in epiphytic plants is very similar to their host, suggesting active bacterial transfer and interaction between epiphytes and their host plants. On the other hand, the bacterial composition in the parasitic plants is very

different from their host, suggesting limited bacterial transfer and interaction between parasitic plants and their host.

Bacterial abundance in parasitic, epiphytic, and host plants

A large number of OTUs are shared between the roots of *Hoya* with their host, and further analysis revealed that bacterial abundance in epiphytes and their host has a positive correlation. For example, the high abundance of 1174-901-12 (uncultured *Rhizobiales*) and *Sphingomonas* in the stem of frangipani also have the same pattern in the root of *Hoya*. Similarly, the high abundance of *Sphingomonas* and *Methylobacterium* in the teak stem is accompanied by the high abundance of these genera in the *Hoya* root (Figure 5). There is no significant difference in bacteria abundance between epiphyte root and their host (T-Test, $p < 0.05$ across all OTUs). Again, these results suggest an active bacterial transfer between the host stem and *Hoya* roots. The bacterial community in epiphytes is highly similar to their host, not only in composition but also in abundance.

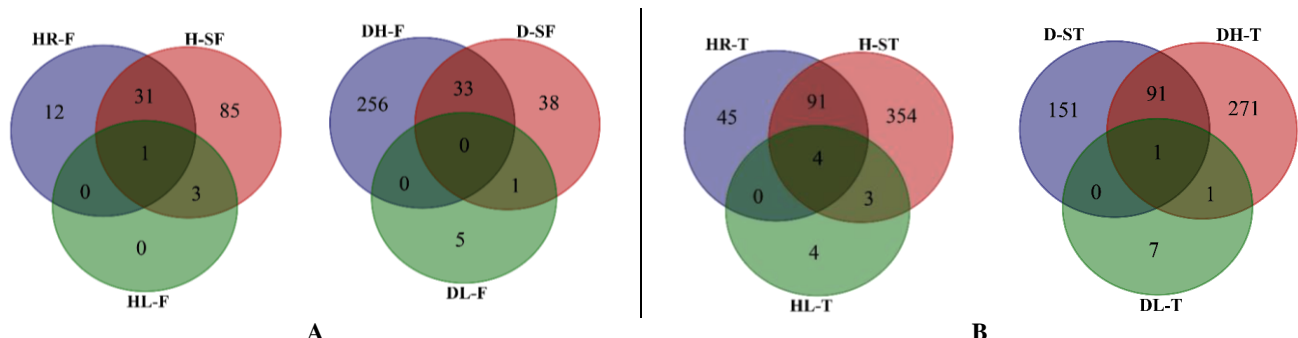


Figure 4. Venn diagram showing the number of shared OTUs between *Hoya* sp. (upper diagram) and *Dendrophthoe* sp. (lower diagram) with the stem of A. frangipani and B. teak. Abbreviations: HL-F: *Hoya* leaf found on frangipani, HR-F: *Hoya* root found on frangipani, H-SF: stem of frangipani hosting *Hoya*, DL-F: *Dendrophthoe* leaf found on frangipani, DH-F: *Dendrophthoe* haustoria found on frangipani, D-SF: stem of frangipani hosting *Dendrophthoe*, HL-T: *Hoya* leaf found on teak, HR-T: *Hoya* root found on teak, H-ST: stem of teak hosting *Hoya*, DL-T: *Dendrophthoe* leaf found on teak, DH-T: *Dendrophthoe* haustoria found on teak, D-ST: stem of teak hosting *Dendrophthoe*

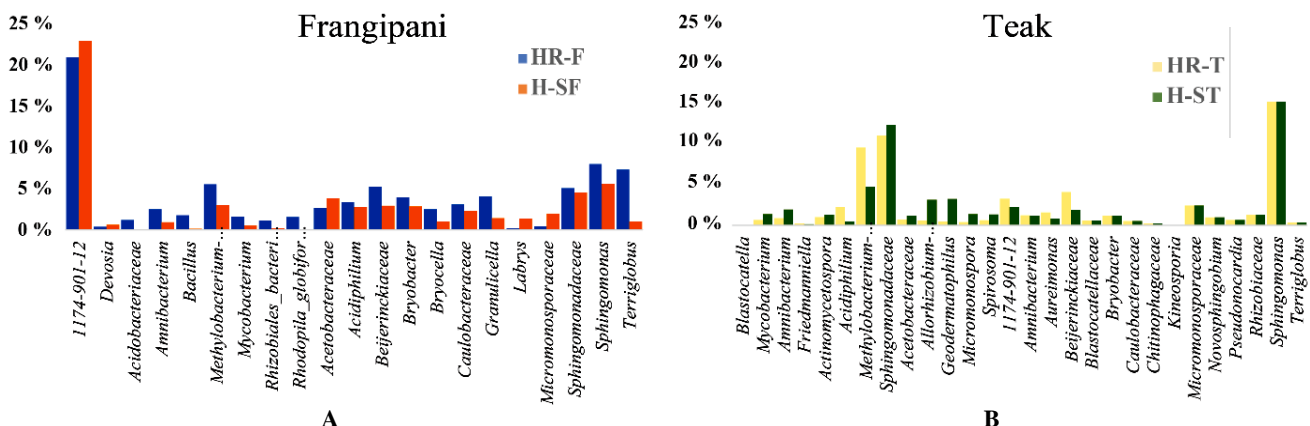


Figure 5. The relative abundance of bacterial taxa is shared between the host plant's stem with the root of *Hoya* sp. A. The relative abundance of bacterial taxa shared between the stem of frangipani with the root of *Hoya* sp. B. The relative abundance of bacterial taxa is shared between the teak stem and the root of *Hoya* sp. Abbreviations: HR-F: *Hoya* root found on frangipani, H-SF: stem of frangipani hosting *Hoya*, HR-T: *Hoya* root found on teak, H-ST: stem of teak hosting *Hoya*

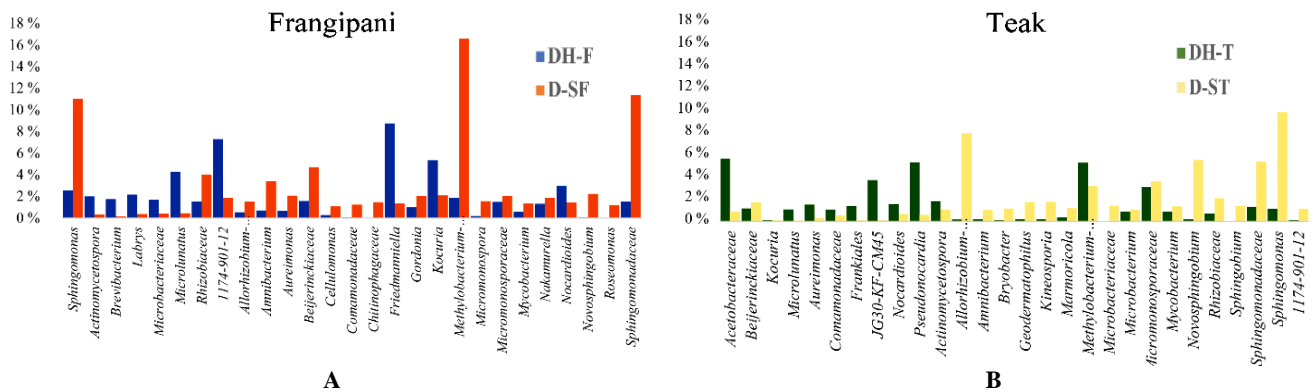


Figure 6. The relative abundance of bacterial taxa is shared between the host plant stem with the haustoria of *Dendrophthoe* sp. A. The relative abundance of bacterial taxa shared between the stem of frangipani with the haustoria of *Dendrophthoe* sp. B. The relative abundance of bacterial taxa shared between the teak stem with the haustoria of *Dendrophthoe* sp. Abbreviations: DH-F: *Dendrophthoe* haustoria found frangipani, D-SF: stem of frangipani hosting *Dendrophthoe*, DH-T: *Dendrophthoe* haustoria found on teak, D-ST: stem of teak hosting *Dendrophthoe*

On the other hand, no correlation was observed between the abundance of bacteria in *Dendrophthoe* haustoria and the stem of the host plants. The high abundance of particular bacteria in the stem of the host is not followed by the high abundance of the same bacteria in *Dendrophthoe* haustoria. For example, *Methylobacterium* is found at high abundance in the stem of frangipani but has a low abundance in *Dendrophthoe* haustoria (T-Test, $p = 0.0256$). Similarly, *Sphingomonas* is abundant in the teak stem but detected low abundance in *Dendrophthoe* haustoria (T-Test, $p = 0.0234$) (Figure 6). In line with previous results, these finding suggests that bacterial composition and abundance in the haustoria of parasitic plants is not correlated with their host.

Discussion

Most studies regarding plant-associated microbiota are conducted in autotrophic-terrestrial plants; however, the formation of bacterial communities in the organ of non-terrestrial epiphytic and parasitic plants is relatively unknown. This study investigates the bacterial communities of the parasitic plant *Dendrophthoe* sp. and epiphytic plant *Hoya* sp. that are associated with two different hosts: Frangipani (*Plumeria* sp.) and Teak (*Tectona grandis*). In epiphytic *Hoya* and parasitic *Dendrophthoe*, only a small number of bacterial OTUs are detected in the leaf (4 to 11 OTUs are identified across different *Hoya* and *Dendrophthoe* leaf samples). Microbes colonize leaves and roots mainly from soil (Fitzpatrick et al. 2018; Chaudhry et al. 2020). *Hoya* and *Dendrophthoe* are attached to the stem or branch of trees that are located a few meters above the ground; lack of access to a bacteria-rich soil environment accompanied by harsh conditions above the ground, such as high exposure to UV radiation and limited nutrient availability might lead to selective pressure that limiting bacterial diversity in the leaf of epiphytic *Hoya* and parasitic *Dendrophthoe* (Herrmann et al. 2021).

The epiphytic *Hoya* roots exhibited high compositional similarity with the host stem microbiome. Regardless of the host species, significant congruency is observed between

Hoya sp. root and host stem microbiome, not only in composition but also in the abundance of shared OTUs. Our results indicate that the bacterial community in the epiphytic plants is mainly derived from and shaped by the host stem microbiota. It is expected since *Hoya* sp. completes its entire life cycle while attached to its host without being connected to the soil. They mainly acquire nutrients from rainwater and the outer surface of the host plant (Rahayu et al. 2007). In the process, microbes might be transferred from the stem of the host plant to the root of *Hoya*.

In contrast, the bacterial community inhabiting *Dendrophthoe* haustoria is quite distinct from its host. Only a small number of OTUs are shared between *Dendrophthoe* with the host stem, and the abundance of the shared OTUs is significantly different. Our results indicate limited bacterial transfer and interaction between *Dendrophthoe* haustoria and the host stem. These results differ from the previously reported parasitic *Orobanchae hederiae* that infect *Hedera* sp. and *Phelipanche aegyptiaca* that infect tomatoes (Iasur Kruh et al. 2017; Fitzpatrick and Schneider 2020). Both studies reported high microbial similarity between the haustoria of parasitic plants with their host. It is worth noting that *Orobanchae hederiae* and *Phelipanche aegyptiaca* are both root parasites, while *Dendrophthoe* is mainly infecting the stem and branches of trees. Parasitic plants obtain nutrients and water from their host using haustoria that penetrate the xylem and phloem tubes in the inner section of the host root or stem system (Yoshida et al. 2016). This process might facilitate bacterial transfer between the two plants; thus, bacterial communities in parasitic plants are probably shaped by microbial pools in the vascular tissue of host plants. In this work, DNA was extracted from the peripheral tissue of the host stem, while in previous studies, bacterial DNA was extracted from the whole infected roots of the host plants. Differences in experimental samples and design might explain our study's and previous studies' differing results. In this study, we found that bacterial composition in *Dendrophthoe* haustoria is distinctly different from the peripheral tissue of the host, but further study is required to evaluate microbial

community in *Dendrophthoe* haustoria is indeed correlated with the microbial pool in the vascular tissue of the host stem, to confirm that bacteria in parasitic plants is mainly derived from the inner section of host organs.

In this study, we demonstrate that although living in the same host plant, epiphytic and parasitic plants regulate and assemble their bacterial communities differently. Epiphytic plants acquire water and nutrients from the outer layer of the host organs. Thus, microbes are transferred from the host plant's surface to the epiphytes' roots. Consequently, the bacterial composition in epiphytes is very similar to the peripheral tissue of the host. On the other hand, parasitic plants develop specialized organs called haustoria that can directly penetrate and absorb nutrients from the inner section of the host organs. This process might transfer microorganisms from the host vascular tissue to the haustoria. Here, we showed that bacterial composition in haustoria is not correlated with the peripheral tissue of the host stem. Further work is required to evaluate whether bacterial composition in haustoria correlates with the host stem's inner section (vascular tissue).

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